Research Description
Cellular, Viral, and Therapeutic Roles of RNA-DNA Hybrids

Maintenance of DNA integrity and sequence is critical for cell survival and propagation with many diseases resulting from failure to replicate or repair damaged DNA. RNA-DNA hybrids, formed by RNA initiation of DNA synthesis, are essential intermediates in DNA replication and repair in cells, and in retroviruses such as HIV. Following their use as primers for DNA replication, RNA-DNA hybrids are normally removed to restore intact DNA molecules by ribonucleases (RNases H) that are specific for RNA in RNA-DNA duplexes. For HIV-1, RNase H activity is an integral part of the enzyme converting the viral RNA into double-stranded DNA prior to integration of DNA into the host's chromosomal DNA. Inactivation of the RNase H renders the virus non-infectious, making this enzymatic activity a target for therapy of this dreaded disease. The structure and enzymatic cleavage mechanism are similar for both viral and cellular proteins making it important to either target the drug specifically to the HIV protein or make certain that inactivation of the cellular enzyme does not lead to unacceptable side effects.

Most organisms have multiple forms of RNase H; two RNases H that are dissimilar in primary amino acid sequence yet have similar structural and, presumably, a common enzymatic mechanism. To address the cellular roles of these RNases H, we have examined the effects of eliminating RNases H in unicellular organisms such as bacteria and fungi. Prokaryotic and eukaryotic cells deleted for either or both RNase H-encoding genes grow quite well but with some important differences from the parental cells. These results suggest that drugs targeted to HIV RNase H need to be examined to see if they provoke effects similar to those found in RNase H-deletion strains (e.g., increased sensitivity to DNA-damaging agents). Examinations of RNase H in mouse and human cells is currently a major focus of our research.

Another important type of RNA-DNA hybrid is that formed by DNA introduced for specific disease treatment. Drug development employing therapeutic DNA oligonucleotides (TOs) is based on the endogenous RNases H for activity. In many diseases, a protein is made at an inappropriate time or location, and elimination of synthesis of the protein would lessen or wipe out the disease. A generic example would be: expression of protein X in heart tissue, an organ in which protein X is usually absent, would lead to major defects in the heart. The messenger RNA for protein X carries the information that specifies the amino acid sequence for the heart tissue to make protein X. The messenger RNA encoding the undesired protein X would be the target for a TO complementary to the mRNA thereby generating a substrate recognized by RNase H initiating degradation of the RNA. There are several clinical trials in progress at the present time (Flaherty *et al.* Curr Opin Oncol 2001:13: 499- 505.) with different targeted mRNAs. Some important features of action of TOs we are examining are: Do both cellular RNases H act to destroy RNAs targeted by TOs? Can the RNase H be modulated in the cell to maximize or in some cases minimize the effectiveness of the TOs? Our experiments using TOs are being carried out in collaboration with Drs. E. Southern and M. Sohail of Oxford University.